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STRUCTURAL CHARACTERISTICS OF PENNISETUM AMERICANUM (PEARL MILLET) USING SCANNING ELECTRON AND FLUORESCENCE MICROSCOPY

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Abstract

Fluorescence, bright field and scanning electron microscopy were used to characterize the structure of selected mature pearl millet caryopses from the World Germplasm Collection. Kernel shape (globose, lanceolate, obovate and hexagonal), kernel endosperm color (white, yellow and grey) and external appearance (color) of the samples were documented for 96 varieties. Color of the pearl millet kernel was due to the combined effects of pigmentation in the pericarp, aleurone and endosperm, as well as the pericarp thickness. White kernels had few pigmented areas, yellow kernels had pigments primarily in the epicarp and endosperm, and brown kernels had pigments in the epicarp, aleurone and endosperm. The majority of white, yellow and brown kernels had a thick pericarp. Purple kernels also had pigments in the epicarp, aleurone and endosperm, but had a thin pericarp. Grey kernels had pigments in the aleurone and endosperm, and had a thin pericarp. The pericarp was different from that found in sorghum in that the epicarp cells could be large, round, multilayered and full of pigments, or flat, single-layered and empty. The seed coat and aleurone layer were similar to those found in sorghum. Phytin and nicotinic acid were present in the germ. β-D-glucans were present in the cell walls in the endosperm.

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KEY WORDS: Pennisetum americanum, pearl millet, fluorescence microscopy, scanning electron microscopy, microstructure, cereal chemistry, cereal quality.

Introduction

The physical and structural properties of pearl millet [Pennisetum americanum (L.) Leeke] vary significantly among varieties (Appa Rao et al., 1985; Rachie and Majmudar, 1980). There are more than 15,000 pearl millet lines in the World Germplasm Collection. While size, shape, germ to endosperm ratio, endosperm texture, pericarp thickness and appearance of the kernel affect processing properties, little information is available to define the variation in structure. In addition, factors affecting pearl millet color are not understood. In contrast, kernel characteristics affecting the color of sorghums and their genetics are clearly understood (Rooney and Miller, 1982). Information on kernel characteristics and its relationship to structure and processing properties of millets would help to improve pearl millet processing quality through breeding and selection.

The structure of pearl millet has been evaluated with scanning and transmission electron microscopy, and bright field microscopy (Badi et al., 1976; Sullins and Rooney, 1977; Adams et al.,1976; Angold, 1979; Zeleznak and Varriano Marston, 1982). In these studies, the research was conducted on a few samples that did not represent the wide variation in kernel properties that exists within the world collection of pearl millet. In general, these studies have suggested that pearl millet structure was similar to that of sorghum kernel structure with two exceptions: pearl millet had no starch in the pericarp, and it had a higher germ to endosperm ratio. Fussell and Dwarte (1980) monitored the development of phenolic compounds in pearl millet with autofluorescence, and found that most of the phenolic compounds were fully developed in the pericarp by 18 days after anthesis. Sullins and Rooney (1977) mentioned that membranous tissue was present between the tube cells and the aleurone layer, but did not classify it as a seed coat. Zeleznak and Varriano-Marston (1982) did not report the presence of a seed coat in pearl millet. Other research has reported that there was a seed coat

Table 1
Kernel characteristics of 96 varieties of pearl millet from 5 different locations[a]

Observation	Location→	India	Niger	Mali83	Mali84	Mali85	Overall
	(n)→	25	15	19	13	14	96
Shape:	obovate	40	46	42	55	72	48
	lanceolate	38	27	21	15	07	25
	hexagonal	11	27	21	15	07	16
	globose	11	00	16	15	14	11
Pericarp:	thick	49	67	58	69	43	55
	thin	51	33	42	31	57	45
Texture:	1 (very corneous)	09	20	16	00	07	11
	2	31	33	37	15	14	28
	3 (intermediate)	20	27	26	54	36	29
	4	20	20	21	31	36	24
	5 (very floury)	20	00	00	00	07	08
Color:	white	14	00	31	00	14	14
	yellow	09	07	11	38	29	16
	brown	17	53	11	08	07	19
	grey	54	40	42	46	50	47
	purple	06	00	05	08	00	04
Pigments Present in Aleurone:		74	69	53	46	43	60
Pigments in Endosperm:							
	none	23	60	26	31	14	29
	yellow	09	40	26	38	29	24
	grey	68	00	48	31	57	47

a) Data are presented as % of n varieties for each location displaying each characteristic; sample size for each variety was 20 kernels.

present in the pearl millet kernel (Narayanaswami, 1953; Rachie and Majmudar, 1980). The germ of pearl millet was very large in proportion to the germ in other cereals (Hoseney et al., 1981; Abdelrahman et al., 1984; Perten, 1983; Reichert and Youngs, 1977), and contained primarily lipids, protein, vitamins and minerals.

The objectives of our research were 1) to describe the structural characteristics found in the majority of pearl millet kernels, 2) to describe the relationship between pearl millet structure and its processing properties, and 3) to provide information on the location of pigmented materials in the kernel that relate to its appearance.

Materials and Methods

Samples

The pearl millet terminology used in this paper is based on that used in Mali, West Africa. A Souna millet is one that matures early in the growing season (less than 100 days), while a Sanio millet is one that matures late (up to 150 days; Bilquez, 1963). The market samples collected in Mali are designated only as Souna or Sanio millets, along with the location of the market, as in Souna Banamba. There are no specific variety names available for these samples as they are composites of many locally grown varieties. Souna and Sanio are also used to define the shapes of millet kernels. In this sense, Souna millets have elongated kernels, while Sanio millets are more globular (round).

Samples of pearl millet were obtained from Pearl Millet Nursuries in Cinzana, Mali (1983, 1984, 1985), Niger (1984) and India (1985). A total of 96 pearl millet varieties and lines were evaluated for pericarp thickness and color, pigmentation in the seed coat, aleurone and endosperm, and the relative proportions of corneous to floury endosperm (texture). The selections were made from the World Collection of Germplasm to represent the widest and most obvious extremes in pearl millet characteristics. Twelve of the most diverse samples were selected for detailed structural evaluation using scanning electron, bright field and fluorescence microscopy. The descriptions in this paper are based on the microscopic examination of these 12 varieties, and unless otherwise noted, these

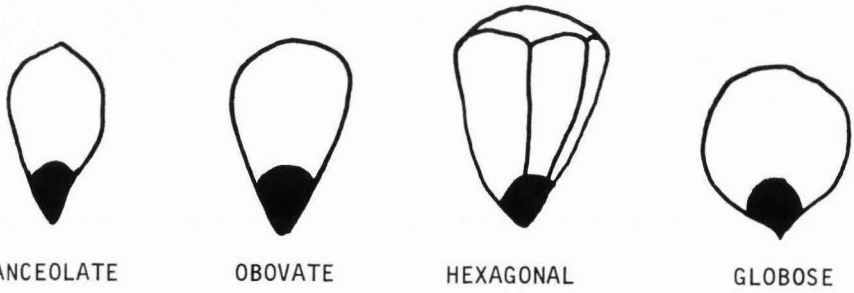


Figure 1: Four primary pearl millet kernel shapes found in 96 samples (IBPGR, 1981).

descriptions are deemed applicable to the entire sample population. Thus no distinctions are made between specific varieties.

Fluorescence Microscopy

The pearl millet samples (6 randomly chosen kernels per variety) were cut in half with a razor blade, fixed in 3.0% gluteraldehyde in 0.025M phosphate buffer (pH 6.8) for 48 hrs, dehydrated in an alcohol series and embedded in glycol methacrylate (Feder and O'Brien, 1968). All samples were sectioned on a rotary microtome (1-2 µm thick) with a glass knife and were viewed on a Zeiss Universal microscope equipped with a IIIRS epi-illuminating system and Zeiss Neofluor objectives.

All sections were stained for fluorescence characterization following the methods outlined by Earp and Rooney (1986), some of which were based on those in Fulcher and Wong (1980). Unstained samples (autofluorescence of ferulic acid and lignin), samples stained with Calcofluor (ß-D-(1-3)(1-4) glucans), and those with ANS (8-anilino-1-napthalene sulfonic acid; protein) were viewed under filter combination (FC) I (exciter filter 365nm, barrier filter > 418nm). Sections stained with acid fuchsin (protein), acriflavine hydrochloride (phytin) and congo red (ß-D-(1-3)(1-4) glucans) were viewed under FC I and III (exciter filter 546nm, barrier filter > 590nm). Sections stained with nile blue A (lipids), diphenylborinic acid (flavonoids) and cyanogen bromide (nicotinic acid) were viewed under FC II (exciter filter 450-490nm, barrier filter > 520nm). Micrographs were taken with Fujichrome 400 film with exposures ranging from 10 sec to 2.5 min.

Bright Field Microscopy

Toluidine blue was used to stain lignin and polyphenolic compounds (O'Brien and McCully, 1981) in the samples (6 randomly chosen kernels per variety) and viewed with a Zeiss Universal microscope equipped with a 100W tungsten light source and Zeiss Neofluor objectives.

Scanning Electron Microscopy

The pearl millet kernels (6 randomly chosen kernels per variety) were cut in half longitudinally with a blunt razor blade, mounted on aluminum stubs with carbon paint, coated with gold-palladium (200Å) and viewed with a JEOL JSM25 scanning electron microscope with an accelerating voltage of 25KV. The dimensions of various kernel structures (starch granules, protein bodies, etc.) were measured using SEM negatives (of known magnification) and a vernier caliper.

Physical and Chemical Analyses

Seventeen samples were analyzed for polyphenol content with the Folin-Ciocalteu assay (Kaluza et al., 1980) and the automated vanillin /HCl method (Maxson and Rooney, 1972; McDonough et al., 1983). Density was determined with a Beckman air comparison pycnometer. Thousand kernel weight was also recorded. Moistures were determined and data were presented on a dry weight basis. Samples were decorticated for 4 min in a TADD mill (Mwasaru, 1985) and the amount of cleaned decorticated sample remaining was considered to be the yield. Three samples, one each of grey (Iniade), yellow (CMM411) and purple (Souna) were prepared for High Performance Liquid Chromatography (HPLC) phenolic acid analysis using the base hydrolysis method of Hahn et al. (1983) and were separated using a 10µm i.d. C-18 column in a Beckman HPLC system.

Results and Discussion

Gross Morphology

The 96 pearl millet varieties in this study represented maximum variation in kernel characteristics (Table 1). The millets exhibited many different shapes (Fig. 1) and colors (IBPGR, 1981). The average profile of a pearl millet variety, based upon observations of all 96 varieties, was an obovate kernel with a thick or thin pericarp, intermediate texture, grey exter-

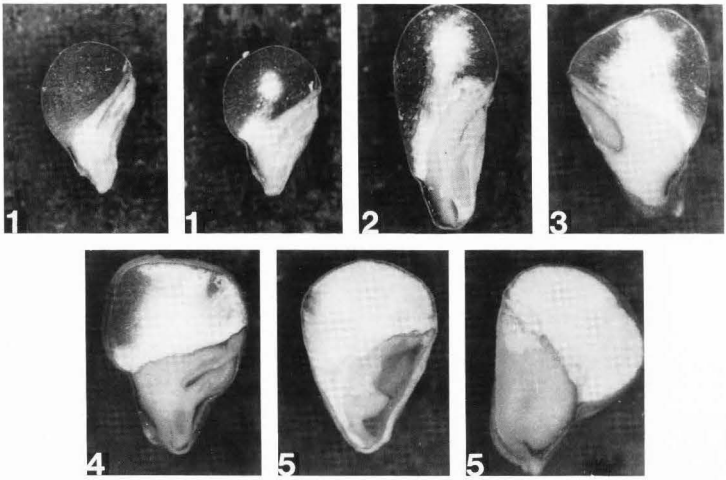


Figure 2: Endosperm texture rating of longitudinal cross sections of pearl millet kernels; 1 is very corneous, 5 is very floury and 3 is intermediate.

Table 2
Physical characteristics of 12 pearl millet varieties chosen for microscopic analysis

Variety	Kernel Shape[a]	Kernel Appearance	Continuous Seed coat[b]	Pigments in Aleurone	Pigments in Endosperm	Pericarp Thickness
Souna	1,2	purple	yes	yes	grey, none	thin
Souna early	2,3	yellow	yes	yes	yellow	thin
Souna Togo	2,3	grey	no	mixture	none	thick
Sanio	2,4	white	yes	no	none	thick
Sikasso	3,4	grey	no	yes	none	thick
CMM424	2	yellow	yes	no	yellow	thin
Cinzana	1	purple	yes	yes	grey	thin
Iniade	2,3	grey	no	yes	grey, none	thin
CMM411	2	yellow	yes	no	yellow	thick
P-13	2,4	yellow, grey	yes	mixture	yellow, grey	thick
GR-P1	2,3,4	yellow	yes	mixture	grey, yellow	thick
Malakondi	2,3,4	grey, brown	no	yes	none	both

a) Ratings: 1- lanceolate; 2- obovate; 3- hexagonal; 4- globose.
b) No grey kernels had a continuous seed coat.

nal appearance, and a pigmented aleurone and starchy endosperm. More specifically, most obovate kernels were yellow or grey, had no pigments in the aleurone, had a pigmented endosperm and mostly corneous texture. Lanceolate kernels were primarily grey, with a pigmented aleurone, grey or yellow endosperm and a variety of pericarp thicknesses and endosperm textures. Hexagonal kernels were similar to the lanceolate ones, except that most had thick pericarps and intermediate to floury texture. Globose kernels were grey with a thick pericarp, pigmented aleurone and a floury white endosperm.

The endosperm texture (Fig. 2) was rated from 1 (very corneous) to 5 (very floury). The majority of the samples had an intermediate texture (rated 2-4). The density of the samples ranged from 1.28 to 1.42 g/cc, with the more corneous kernels having the highest density

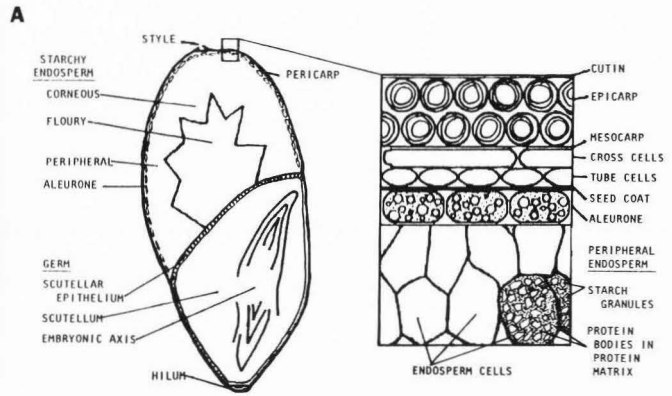
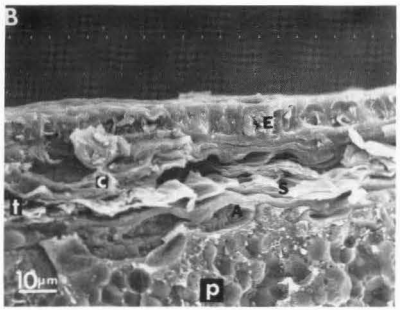
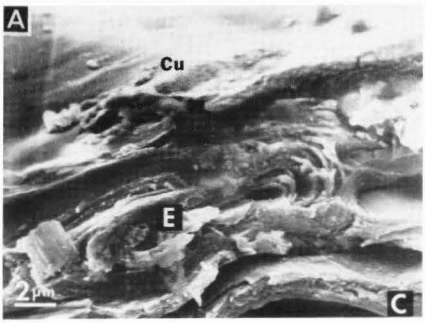


Fig. 4 is a color plate on pp. 143.





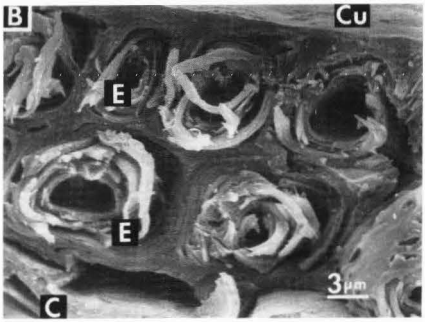


Figure 3: A. Overall structure of the endosperm, germ and pericarp of a pearl millet kernel; B. Cross-section of the pericarp and peripheral endosperm of a Souna pearl millet. E: epicarp cell, C: cross cell, t: tube cell, s: seed coat, A: aleurone cell, p: peripheral endosperm.

Figure 5: A. Cutin layer and epicarp cell in the pericarp of a purple Souna variety; B. Double epicarp layer with pigmentation in the epicarp cells of a purple Souna variety. Cu: cutin layer, E: epicarp cell, C: cross cell.

values. The 1000 kernel weight ranged from 2.5 to 20.0 g. The very corneous varieties generally had small kernels.

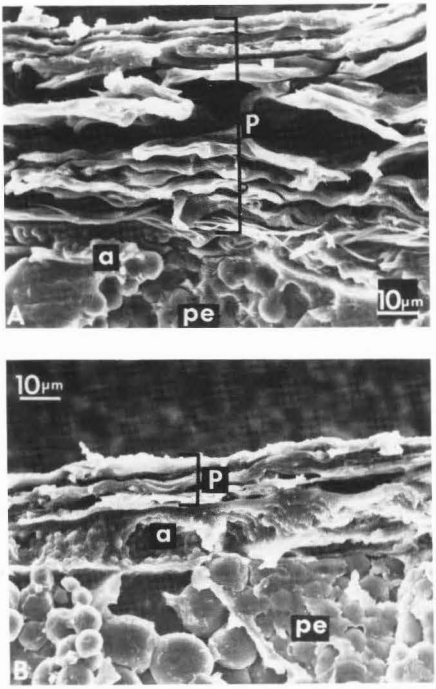


Figure 6: Two varieties of pearl millet (varieties unknown) with thin epicarp layers that illustrate the difference in pericarp thickness that can occur due to a difference in mesocarp thickness. A. Thick pericarp variety; B. Thin pericarp variety. P: Overall thickness of the pericarp (all structures included), a: aleurone cell, pe: peripheral endosperm cell.

Pericarp

The structural descriptions presented here are based on the detailed study of the 12 diverse varieties previously mentioned (Table 2). The descriptions are consistent, and can be applied to the vast majority of the 96 varieties. Exceptions to this are noted.

The tissues in the pericarp are shown in Figs. 3 and 4A. The pericarp was composed of the epicarp, mesocarp and endocarp layers. The epicarp was usually one to two layers thick, with large blocky cells that contained concentric layers of pigmented tissue (Figs. 4A, 5). However, some varieties of pearl millet had long, narrow, flat epicarp cells with no apparent cell contents (Fig. 6). This demonstrates the varietal differences that occur, since all of the varieties pictured in Fig. 5 and 6 are Souna types. Rachie and Majmudar (1980) reported that some pearl millet varieties (unspecified) had pericarps with flat, empty epicarp cells. A cutin layer covered the outside of the epicarp layer; the cutin stained positive with nile blue A (Fig. 4B).

The mesocarp layer was directly beneath the epicarp and contained several layers of compressed cells that were often indistinguishable from the cross and tube cells (Fig. 6). Frequently it was not possible to distinguish individual cell walls in this layer, due primarily to compression of the cells during grain maturation. The overall thickness of the pericarp could be due to the number of cell layers present in the mesocarp or to the presence of a thick or thin epicarp. There were no starch granules present in the pericarp in any pearl millet variety, contrary to what has been reported in sorghum (Earp, 1984).

Beneath the mesocarp were the cross and tube cells, or endocarp (Fig. 4A), which may be responsible for nutrient and moisture transport around the developing kernel (Rooney and McDonough, 1987). Cross cells were oriented perpendicular to the long axis of the kernel. The tube cells were perpendicular to the cross cells.

There was a seed coat present beneath the endocarp that was observed in all 12 varieties studied; it measured 0.4µm in thickness (Fig. 7). The seed coat appeared to be lightly pigmented, but no distinct cells containing pigments were observed, and it bore little resemblance to the heavily pigmented seed coats found in some sorghum varieties (Earp and Rooney, 1986). The seed coat was continuous in most varieties, with the exception of approximately half of the grey varieties, in which its presence corresponded with the areas that were grey in color. These varieties included both Souna and Sanio type millets. A possible explanation for this may be that pearl millet can have a partial testa similar to that reported in sorghum by Blakely et al. (1979).

When several varieties of pearl millet were decorticated in a TADD mill, the pericarp split from the kernel just beneath the endocarp, leaving the aleurone intact. This agreed with the results of Sullins and Rooney (1977); however, de Francisco et al. (1982) reported that the pericarp split away from the kernel below the aleurone layer. The differences could be attributed to decortication time or method. Decortication characteristics are important in food processing, since the aleurone contains protein, vitamins and minerals that enhance the nutrient value of prepared food. Globose kernel shapes (Sanio-type millets) are more useful under average traditional decortication conditions; if the kernels have a thick pericarp, they can be decorticated with a minimum loss of starchy

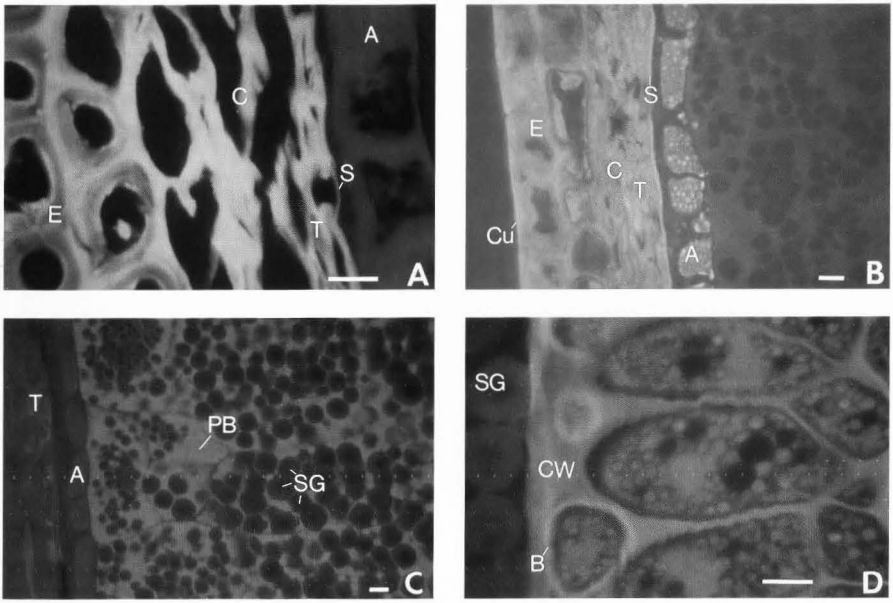


Figure 4: Fluorescence micrographs of pericarp, endosperm and scutellar epithelium of pearl millet. A. Autofluorescence of the pericarp in a purple Souna variety; B. Nile blue A staining of lipids in the pericarp and aleurone of a yellow Souna variety; lipids in the endosperm were extracted during dehydration; C. ANS staining of protein in the peripheral and outer corneous endosperm areas of a yellow Souna variety; D. Congo red stain viewed with FC I showing ß-glucans in the cell walls of the scutellar epithelium in Iniade (grey variety); starch granules appear red in the floury endosperm. Bar = 4µm. Cu: cutin layer, E: epicarp cell, C: cross cells, T: tube cells, S: seed coat, A: aleurone cell, PB: protein bodies, SG: starch granule, B: ß-glucan material, CW: cell wall.

endosperm (Coulibaly and Kante, 1983). Globose kernels were decorticated more effectively in this study than hexagonal or lanceolate kernels; the more elongated kernels tended to break in half during decortication and yield was very low.

Aleurone Layer

The aleurone layer was beneath the seed coat, and was one cell layer thick (Fig. 3B). The cell walls were very thick, and fluoresced a deep royal blue under FC I (Fig. 4A); the blue color appeared darker than sorghum aleurone examined under the same conditions Earp (1984). Lipids were visible as small yellow bodies when stained with nile blue A (Fig. 4B) and were found throughout all aleurone cells. Rachie and Majmudar (1980) indicated that the aleurone contained primarily lipids, protein, phytin and occasionally pigments, which added to the overall color perception of the kernel. Lai and Varriano-Marston (1980) reported that there were high levels of lipids in the aleurone.

Starchy Endosperm

The starchy endosperm of pearl millet was composed of peripheral (or subaleurone), corneous and floury areas (Fig. 8). These three areas have already been documented in sorghum (Earp, 1984), corn (Wolf et al., 1952) and pearl millet (Sullins and Rooney, 1977). The cells were small in the peripheral endosperm (21 x 40µm) and larger in the corneous and floury endosperm (73 x 83µm).

The peripheral endosperm was 1-3 cell layers thick, had polygonal starch granules embedded in a thick protein matrix, and contained a large number of protein bodies. The peripheral endosperm cell contents were

Table 3
Factors that contribute to the external appearance of pearl millet kernels

Seed color	n[a]	Pigments in Epicarp	Pericarp Thickness[b]	Pigments in Aleurone[b]	Pigments in Starchy Endosperm[b]
White	13	no	thick (69.2)[c] thin (30.8)	none (38.5) grey (38.5) yellow (23.0)	absent (61.5) present (38.5)
Yellow	15	yes	thick (66.7) thin (33.3)	yellow (80.0) grey (13.3) none (6.7)	absent (93.3) present (6.7)
Brown	18	yes	thick (77.8) thin (22.2)	none (44.5) yellow (33.3) grey (22.2)	present (61.1) absent (38.9)
Purple	4	yes	thin (100.0)	grey (100.0)	present (100.0)
Grey	46	no	thin (56.5) thick (43.5)	grey (65.2) none (30.4) yellow (4.4)	present (82.6) absent (17.4)

a) Number of cultivars within each color group.
b) 20 seeds of each variety were hand-dissected; varieties were categorized according to the characteristics observed in the majority of the 20 seeds.
c) Percent of the cultivars in that color group displaying each attribute.

packed tightly together and the protein bodies left distinct indentations in the starch granules. The average sizes of a starch granule and a protein body were 6.4 and 0.7μm, respectively. The protein to starch ratio was highest in the peripheral endosperm layers (Fig. 4C).

The corneous and floury endosperm comprised the bulk of the starchy endosperm; the relative amount of each depended on the genotype. The corneous endosperm was composed of cells that were packed with starch granules and a thin, semi-continuous protein matrix. Protein bodies were also present, but in fewer numbers than in the peripheral endosperm. Generally, the corneous endosperm cell contents were not packed tightly enough for the protein bodies to leave indentations in the starch granules. There were no air voids between the granules, which were less polygonal than those found in the peripheral areas; this gave the corneous endosperm a glossy appearance. Starch granules and protein bodies averaged 6.4 and 0.7μm in diameter, respectively.

The floury endosperm was composed of cells with loosely packed, larger, round starch granules with a small amount of discontinuous protein matrix. There were many air voids between the starch granules, which gave the floury endosperm a chalky appearance. There were few protein bodies present, and thus no indentations in the starch granules. The sizes of the starch granules and protein bodies averaged 7.6 and 0.6μm, respectively.

Protein

The highest amount of protein was found in the peripheral endosperm and decreased from the exterior to the interior of the kernel (blue fluorescence in Fig. 4C). Hoseney and Varriano-Marston (1980) reported that there were no protein bodies found in the floury endosperm of pearl millet; however, there were protein bodies present in the floury endosperm of all of the varieties observed in this study. The protein bodies were spherical and roughly uniform in size, regardless of their location in the endosperm. Adams et al., (1976) reported that the protein bodies contained invaginations and protuberances, and were not uniform in shape. The protein bodies seen in this study were somewhat smaller than those reported by Sullins and Rooney (1977) and Zeleznak and Varriano-Marston (1982).

A considerable amount of the protein in the pearl millet kernel was found in the protein bodies of the germ, as has been reported previously in many studies. All of the millets examined contained phytin in the germ. Phytin is important due to its interference in the bioavailability of minerals. Simivemba et al., (1984) reported that phytic acid was present in the germ and pericarp, but that the content varied greatly between environmental locations. Nicotinic acid inclusions were present in the protein bodies of the germ, but none were found in the protein bodies of the aleurone cells.

Lipids

Pearl millet has a lower endosperm to germ ratio than sorghum (Abdelrahman et al., 1984; Hoseney and Varriano-Marston, 1980). The germ contained a large proportion of the lipids found in the kernel. As previously reported, there was also a high concentration of lipids in the aleurone cells. Small globules of lipid were distributed throughout the endosperm cells of fresh hand sectioned material stained with Nile blue A (not shown); no lipids were visible in samples that had been fixed and dehydrated.

ß-Glucans

The aleurone cell walls in pearl millet autofluoresced bright blue (Fig. 4A). The endosperm cell walls exhibited weak autofluorescence (not shown). Earp et al., (1983) reported that ferulic acid was responsible for bright blue fluorescence in the pericarp, aleurone and endosperm cell walls of sorghum. Fussell and Dwarte (1980) used autofluorescence to find that pericarp cells associated with black region development in pearl millet were composed of lignin.

When stained with congo red and viewed under FC I, mixed linkage ß-glucan material was located in the cell walls of the scutellar epithelium; the ß-glucan material appeared red along the inside of the cell walls around each cell (Fig. 4D). Fulcher and Wood (1983) reported that the red fluorescence under FC I was due to mixed linkage ß-D-glucans. Congo red induced red fluorescence in ß-D-(1-3)(1-4) glucans in cell walls of the pericarp, endosperm and germ, when viewed under FC III, but no differentiation between ferulic acid and ß-glucans was possible using this filter combination.

Role of Pigmentation in External Kernel Color

Pigmentation imparts positive or negative attributes to food products, and in many areas of Africa, foods with a light color are preferred. Thus, it is important to know where the pigments are, what they are, and if they can be removed. In the 96 pearl millet samples studied, the external color perceived for each kernel was due to the interaction of several factors: pericarp thickness, pigmentation in the epicarp, slight pigmentation in the aleurone, and the existence of unidentified pigmentation in the peripheral endosperm (Table 3).

Pericarp: The epicarp cells contain a considerable amount of pigmentation in some varieties; the structure has been described previously. A thick pericarp can mask the presence of pigments in the aleurone or endosperm, which was observed in several white varieties. However, when the pericarp is thin, the pigmentation in the aleurone and endosperm is visible, and the external color of the seed can be yellow, brown, purple or grey. If there are no pigments present in the kernel, and the pericarp is thin, then the color of the kernel is white.

If pigmentation is present in the seed coat, it does not have a great deal of effect on the external color perception of the kernel because the layer is so thin. In contrast, the seed coat (testa) found in sorghum with B_1-B_2- genes can be heavily pigmented in discreet cells, which definitely influences the external color per-

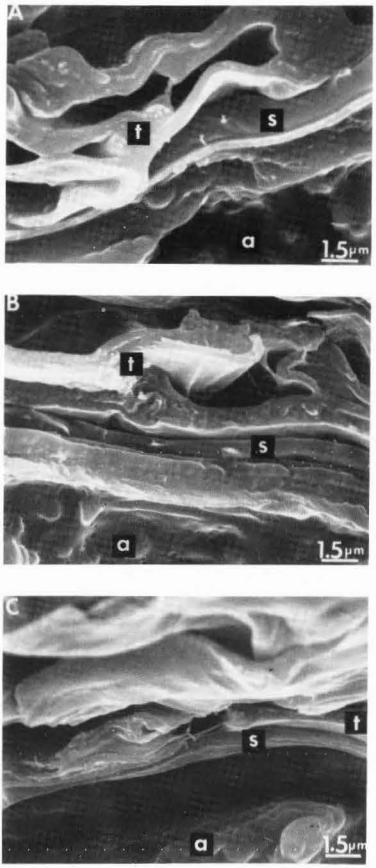


Figure 7: Cross-sections of the endocarp layers of three pearl millet varieties showing the presence and thickness of the seed coat. A. Yellow Souna variety; B. Purple Souna variety; C. Grey Iniade variety; in this and other grey varieties, the seed coat was present underneath the areas with a grey external color. t: tube cell, s: seed coat, a: aleurone cell.

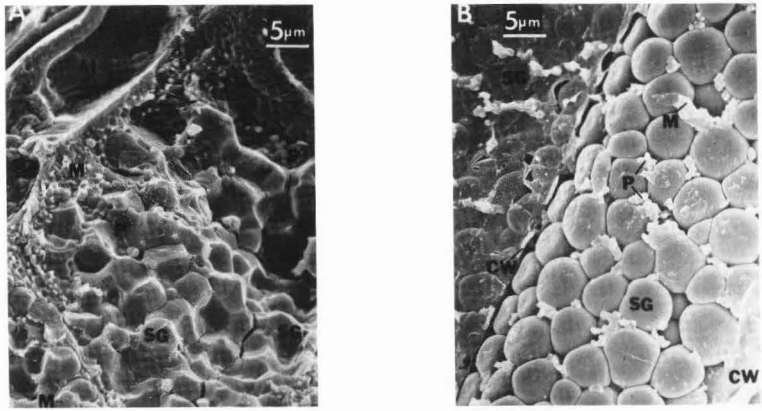


Figure 8: Starchy endosperm areas of an intermediate texture grey Souna variety. A. Peripheral endosperm area with dense protein matrix and a large number of protein bodies; B. Corneous endosperm with fewer protein bodies and thin protein matrix; C. Floury endosperm with little protein matrix and a few scattered protein bodies. Al: aleurone cell, M: protein matrix, P: protein body, SG: starch granule, CW: cell wall.

ceived in the kernel (Earp, 1984). It is easier to obtain an acceptable product color in foods when the pigmentation is primarily in the pericarp, where it will be removed during decortication.

Aleurone: Polyphenols were found in the aleurone cells after staining with toluidine blue; a pale green color resulted (not shown). Pigmentation in the aleurone is dark; it has the greatest effect on external color in varieties with a thin pericarp. This is demonstrated most clearly in the grey and purple millets. The aleurone did not stain positive for flavonoids (diphenylborinic acid), but the compounds may have been extracted during the dehydration process.

Endosperm: Yellow endosperm was observed most frequently in the yellow varieties, and to a lesser extent in brown and white varieties. A very small percentage of grey varieties had a yellow endosperm. The yellow color was most evident in the corneous endosperm. Rachie and Majmudar (1980) reported that ß-carotene in the endosperm can cause the external color of the kernel to appear yellow in seeds with a colorless seed coat and a thin unpigmented pericarp.

Grey pigments were observed in the peripheral and corneous endosperm of all purple, and most grey varieties, and in a small percentage of yellow and brown varieties. There were an equal number of white varieties that contained grey pigments and those that contained no pigments, but usually these varieties had a thick pericarp. When endosperm sections were viewed under FC I (autofluorescence), there were often small patches of dark pigmentation located within the peripheral and corneous endosperm cells (not shown).

External Color: It is difficult to say if a specific kernel characteristic produces a specific kernel color. Rather, it is the combination of kernel characteristics that result in specific colors. However, there are a number of possible combinations. In varieties with a pigmented aleurone, the color could be dark with a thin pericarp, or light with a thick pericarp. Likewise, a dark endosperm could produce a grey tint in a white or yellow millet with a thick peri-

carp. A dark endosperm in a thin pericarp variety can result in grey or purple color. A yellow endosperm can enhance the yellow color in some varieties with a thin pericarp, or have no effect on the external color in varieties with a pigmented aleurone and pericarp. If pigments are in the epicarp, the external color is most likely going to be the color of those pigments. However, there are often cases where the varieties have bicolored kernels, i.e., grey and brown, or grey and yellow, where there appear to be concentrations of pigmentation in the base and tip of the kernels, with grey color in the midrange. There was a uniform set of kernel characteristics only in the purple millet varieties; a pigmented aleurone and endosperm, a thin pericarp, and dark pigments in the epicarp resulted in the purple color.

Polyphenol Analyses

The phenol analyses of 17 pearl millet varieties revealed that there were levels of polyphenols present in all samples tested. A purple variety had 0.33 mg/ 100mg polyphenols, while grey and yellow varieties averaged 0.22 and 0.19 mg/100mg, respectively. None of the samples contained tannins, which agreed with results previously reported by Reichert (1979). Reichert (1979) reported that the pigments in the grey pearl millet varieties were composed of C-glycosylflavonoids.

The HPLC analyses of three varieties of pearl millet revealed high levels of ferulic, coumaric, cinnamic and gentisic acids (Table 4). There were differences in phenolic acid content evident between pericarp colors. Total phenolic acid levels were highest in the yellow millet, followed by the grey and purple millets.

Table 4

Phenolic acid analyses of 3 pearl millets[a]

Phenolic Acids	Purple (Souna)	Grey (Iniade)	Yellow (CMM411)
Ferulic	624.7	786.3	628.2
Coumaric	247.8	211.4	346.6
Gentisic	144.2	79.0	96.0
Cinnamic	271.4	350.1	415.1
Caffeic	11.3	37.5	15.1
Vanillic	16.3	26.1	6.5
p-OH Benzoic	24.1	15.8	26.0
Syringic	17.8	10.5	23.7
Sinapic	18.4	15.4	27.7
Unknowns	669.0	646.5	892.8
Total Acids	2037.7	2182.4	2486.6

a) values expressed as µgm phenolic acids/ gm sample, dry weight basis; seed from locations in Mali, West Africa; values are the averages of two replicates each.

Conclusions

The data presented in this study provide some insight into the relationship between kernel characteristics, kernel structure, and processing properties of pearl millet. The diverse array of external characteristics and the lack of genetic information makes it difficult to predict how individual varieties of pearl millet will behave during processing. More knowledge of the kernel structure and kernel characteristics affecting processing properties is required. This study provides a starting point for more definitive work in the future.

Acknowledgements

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Discussion with Reviewers:

SH Yiu: Is the textural difference of the starchy endosperm, i.e. corneous v. floury, dependant on the variety or the maturity of the kernel of pearl millet?

Authors: The texture of the endosperm depends upon both variety and environmental factors. A corneous variety can develop a floury endosperm when it is affected by insects, grain molds and weathering. A floury endosperm variety never develops a corneous texture.

SH Yiu: The negative results obtained from staining with diphenylborinic acid may suggest a low concentration of flavonoid compounds or removal of these compounds by alcohols (F.W. Collins, 1986. In "Oats: Chemistry and Technology, AACC, St. Paul, Minnesota) that can occur at the dehydration step during preparation of the glycol methacrylate embedded sections. Did you try staining using hand-prepared or frozen sections?

Authors: Upon this suggestion, fresh sections were prepared by hand and stained with diphenylborinic acid. Some very weak fluorescence was evident in a purple Souna millet, but none appeared in the grey or yellow millets. The dehydration process does seem to be inhibiting the response to diphenylborinic acid, but to what extent is unknown.